

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

Applicant's or agent's file reference	<b>FOR FURTHER ACTION</b>		See Form PCT/IPEA/416
International application n° PCT/FR2005/000479	International filing date (day/month/year) 28.02.2005	Priority date (day/month/year) 27.02.2004	
International Patent Classification (IPC) or both national classification and IPC  INV. C12N1/02 C12M1/12 A23L1/30 A23L2/52 A23C9/152			
Applicant :  COMPAGNIE GERVAIS DANONE et al.			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> (sent to the applicant and to the International Bureau) a total of <u>  4  </u> sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the report</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input checked="" type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand of international preliminary examen		Date of completion of this report:	
15.12.2005		07.06.2006	
Name and postal address of authority conducting international preliminary examination European patent office D-80298 Munich Tel : +49 89 2399 - 0 Tx: 523656 epmu d Fax : +49 89 2399 - 4465		Authorized officer  Espen, J  Telephone n° : +49 89 2399 - 8410	

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application n°  
PCT/FR20045/000479

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**Box No. I Basis of the opinion**

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1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☒ This opinion is based on a translation from the original language into the following language, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1 (b))
  - ☐ publication of the international application (under Rule 12.4)
  - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the elements\* of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

the description, pages

1-19 as originally filed/furnished

the claims, No.

1-28 received on 15.12.2005 accompanied by letter dated 08.12.2005

the drawings, sheets

1/5-5/5 as originally filed/furnished

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to the sequence listing (*specify*):
4. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to the sequence listing (*specify*):

\* If item 4 applies, some or all of those sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application n°  
PCT/FR2005/000479

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**Box No. V Reasoned statement under Rule 35.2, with regard to novelty, inventive step  
or industrial applicability; citations and explanations supporting such  
statement**

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1. Statement

Novelty	Yes:	Claims	1-20, 24-28
	No:	Claims	21-23
Inventive step	Yes:	Claims	1-20, 24-28
	No:	Claims	21-23
Industrial applicability:	Yes:	Claims	1-28
	No:	Claims	-

2. Citations and explanations (Rule 70.7) :

**see separate sheet**

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**Box No. VIII Certain observations on the international application**

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The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**WRITTEN OPINION OF THE INTERNATIONAL  
PRELIMINARY EXAMINING AUTHORITY  
(SEPARATE SHEET)**

International application N°

**PCT/FR2005/000479**

**Concerning point V**

**Declaration motivated as to novelty, inventive activity and the possibility of industrial application; citations and explanations in support of this declaration**

Reference is made to the following documents:

- D1: CRESPO, J. P. S. G. et al: "Tangential flow filtration for continuous cell recycle culture of acidogenic bacteria" CHEMICAL ENGINEERING SCIENCE, 47(1), 205-14 CODEN: CESCAC; ISSN: 0009-2509, 1992, XP009034901
- D2: MAUS J E et al: "Employment of stressful conditions during culture production to enhance subsequent cold- and acid-tolerance of bifidobacteria." JOURNAL OF APPLIED MICROBIOLOGY, vol. 95, no. 1, 2003, pages 146-154, XP002334925 ISSN: 1364-5072
- D3: HAYAKAWA K et al: "High Density Culture of Lactobacillus casei by a Cross-Flow Culture Method Based on Kinetic Properties of the Microorganism" Journal Of Fermentation And Bioengineering, vol. 70, no. 6, 1990, pages 404-408, XP002335665 ISSN: 0922-338X

Document D1 describes a device which is suitable for carrying out a process for production of a liquid concentrate of adapted and viable bacteria for foodstuff usage in that it comprises a vat containing a washing solution (feed vessel), an inlet conduit of said solution in a fermenter (reactor), an outlet conduit to convey the culture medium containing the bacteria to two tangential microfiltration modules. Each module has a membrane of total surface of 0.1 m<sup>2</sup>. Said modules enable separation of said culture medium into a permeate containing no bacteria and a concentrate containing bacteria. In addition, D1 divulges a device characterised in that the concentrate is recycled on leaving the tangential microfiltration modules by reincorporation into the fermenter.

Document D2 describes a production process of adapted bifidobacteria, where adaptation is disclosed by measuring parameters of the culture medium and/or parameters of the bacteria (D2, p. 148, left column). In light of D2 (p. 148), the bacteria resulting from this process are adapted to an acid (pH) medium and reduced temperature. Further, D2 describes a yoghurt additive, characterised in that the food additive comprises the abovementioned adapted and viable bifidobacteria.

In addition, D2 mentions that following 2.5 to 5 hours of exposure of bacteria of the genre *Bifidobacterium lactis* to the yoghurt, there is no significant difference with respect to tolerance to acidity between the adapted and the non-adapted bacteria (D2, p. 152-153).

Document D3 describes a process for production of a liquid concentrate of bacteria, for food usage, comprising the following successive steps:

- a) the bacteria are propagated in a fermenter in an appropriate culture medium;
- b) this is diluted with fresh culture medium at a rate corresponding to the concentration of the cellular mass;
- c) the medium containing the bacteria is concentrated in bacteria by tangential microfiltration (cross-flow) until a bacterian concentration of  $11^{11}$  ufc/ml is reached;
- d) a liquid concentrate of bacteria for food usage is recovered.

In particular, the above process is conducted with the aim of removing inhibiting metabolites.

1). The present application does not fulfil the conditions stated in article 33 (1) PCT, the object of Claims 21-23 not conforming to the criterion of novelty defined by article 33 (2) PCT.

1.1). In light of the remarks made hereinabove, document D1 describes a device having all the technical characteristics of Claims 21-23. As a consequence said device is suitable for carrying out a process for production of a liquid concentrate of adapted and viable bacteria for food usage.

Therefore, the object of Claims 21-23 is not novel.

2). In light of the above documents, it seems that the object of Claim 1-20 and 24-28 formally fulfils the conditions stated in article 33 (2) PCT.

3). The solution proposed in Claims 1-20 and 24-28 of the present application is considered as implying inventive activity (article 33(3) PCT), and for the following reasons:

3.1). Document D2 is considered as being the closest state of the art. In light of the comments made hereinabove, the process of Claim 1 differs from the content of D2 in that the culture medium containing the bacteria adapted by tangential microfiltration is washed until a bacterian concentration greater than  $5 \times 10^{10}$  and advantageously greater than  $1 \times 10^{11}$  ufc/ml is obtained.

3.2). The problem raised to resolve the present invention can thus be considered as being to enrich the state of the art with a liquid concentrate of active and viable bacteria for food usage.

3.3). The specialist wanting to carry out production of a liquid concentrate of adapted and viable bacteria would not have considered document D3, which discloses washing of bacteria by tangential microfiltration with the aim of removing unwanted metabolites from the culture medium.

3.4). Consequently, the object of Claims 1-20 and 24-28 could not have been realised evidently.

### **Concerning point VIII**

#### **Certain observations relative to the international application**

The set of claims does not fulfil the conditions stated in article 6 PCT.

1). In light of page 2 of the description, it seems that the tangential filtration step under certain conditions allows the desired volumes of bacteria culture to be concentrated, at the same time preserving their viability and without clogging the filters.

Said particular conditions are considered as being characteristics essential to the realisation of the present international application.

2). The liquid concentrate of adapted and viable bacteria (Claim 24) and the additive food product (Claim 26) are completely deficient in light of article 6 PCT, since they comprise no true technical characteristic which would allow to it to be distinguished from disclosures made in D2.

CLAIMS

1. A production process for a liquid concentrate of adapted and viable bacteria, for use in foodstuffs comprising the following successive steps:

a) the bacteria are propagated in a fermenter in an appropriate culture medium;

b) the bacteria obtained are adapted to step a);

c) the culture medium containing the bacteria adapted by tangential microfiltration is washed using a washing solution;

d) the washed medium containing the bacteria adapted by tangential microfiltration to a bacterial concentration greater than  $5 \cdot 10^{10}$  ufc/ml advantageously greater than  $1 \cdot 10^{11}$  ufc/ml are concentrated in bacteria;

e) a liquid concentrate of adapted and viable bacteria for use in foodstuffs is recovered.

and/or adaptation of the bacteria carried out at step b) is disclosed by measuring parameters of the culture medium and/or bacteria parameters.

2. The process as claimed in Claim 1, characterised in that the bacteria are lactic bacteria, in particular bacteria of *Lactobacillus* spp, *Bifidobacterium* spp., *Streptococcus* spp and *Lactococcus* spp genera.

3. The process as claimed in Claim 1 and 2, characterised in that the culture medium of step a) is a synthetic medium.

4. The process as claimed in any one of the preceding claims, characterised in that the culture medium containing the bacteria in the fermenter at the end of step a) has a pH between 3 and 6.

5. The process as claimed in any one of the preceding claims, characterised in that the concentration of bacteria at the end of propagation step a) is greater than  $2 \cdot 10^{10}$  ufc/ml.

6. The process as claimed in Claim 1, characterised in that the parameters of the culture medium are the pH, the osmotic pressure and/or the temperature of the culture medium.

7. The process as claimed in Claim 6, characterised in that the parameter of the culture medium is the pH and in that the step b) is taken by reducing the pH by natural acidification.

8. The process as claimed in Claim 6, characterised in that the parameter of the culture medium is the temperature, and in that step b) is taken by reducing the temperature.

9. The process as claimed in any one of Claims 1, 6, 7, 8, characterised in that the parameter of the bacteria is the size of the bacteria.

10. The process as claimed in Claim 1, characterised in that the distribution of the lengths of each bacterium is predominantly between 0.1 and 10 micrometres, advantageously between 0.5 and 5 micrometres.

11. The process as claimed in any one of the preceding claims, characterised in that adaptation step b) is taken by tangential microfiltration.

12. The process as claimed in any one of the preceding claims, characterised in that the tangential microfiltration membranes have a porosity between 0.01 and 0.5  $\mu\text{m}$ , advantageously between 0.1 and 0.3  $\mu\text{m}$ .

13. The process as claimed in any one of the preceding claims, characterised in that in step c) the inlet pressure of the culture medium in the microfiltration module is between 0 and  $3 \cdot 10^5$  Pa.



14. The process as claimed in any one of the preceding claims, characterised in that in steps c) and d) the rate of the permeate is between 0.001 and 0.1 m<sup>3</sup>/h/m<sup>2</sup> of surface exchange.

15. The process as claimed in any one of the preceding claims, characterised in that in step d) the transmembrane pressure is between 0.1.10<sup>5</sup> and 2.10<sup>5</sup> Pa and advantageously between 0.1.10<sup>5</sup> and 0.5.10<sup>5</sup> Pa.

16. The process as claimed in any one of the preceding claims, characterised in that in step d) the recirculation rate of the washed medium is between 0.5 and 3 m<sup>3</sup>/h/m<sup>2</sup> of exchange surface and advantageously between 0.8 and 1.25 m<sup>3</sup>/h/m<sup>2</sup> of exchange surface.

17. The process as claimed in any one of the preceding claims, characterised in that it comprises prior to step a) successive steps of revival and preculture of the bacteria.

18. The process as claimed in any one of the preceding claims, characterised in that it comprises an additional step f), following step e), of packaging the liquid concentrate of adapted and viable bacteria in flexible and hermetic bags.

19. The process as claimed in Claim 18, characterised in that it comprises an additional step g), following step f), of keeping the liquid concentrate of adapted and viable bacteria packaged in flexible bags and hermetic at a temperature between -50°C and +4°C.

20. The process as claimed in Claim 19, characterised in that it comprises an additional step h), following step g), of reheating by adapted means of the liquid concentrate of adapted and viable bacteria packaged in flexible and hermetic bags.

21. A device for executing the process for production of a liquid concentrate of adapted and viable bacteria for use in foodstuffs as claimed in any one of Claims 1 to 20, characterised in that it comprises a vat (1) containing a washing solution, an inlet conduit (2) of said washing solution in an fermenter (3), said fermenter (3) serving as propagation of the bacteria in a culture medium, an outlet conduit (4) for conveying the culture medium containing the bacteria to one or more modules (5) of tangential microfiltration, said modules (5) allowing separation of said culture medium into a permeate (6) not containing bacteria and into a concentrate (7) containing the bacteria.

22. The device as claimed in Claim 21, characterised in that the concentrate (7) is recycled on leaving the filtration modules (5) by reincorporation into the fermenter (3).

23. The device as claimed in Claims 21 and 22, characterised in that the filtration modules (5) comprise from 1 to 10 filtration membranes, each membrane representing from 0.1 m<sup>2</sup> to 150 m<sup>2</sup> of total filtration surface.

24. A liquid concentrate of adapted and viable bacteria, characterised in that it is likely to be obtained by the process as claimed in any one of Claims 1 to 20.

25. Utilisation of the liquid concentrate of adapted and viable bacteria as claimed in Claim 24 as a foodstuff additive.

26. An additive food product, characterised in that the foodstuff additive utilise is a liquid concentrate of adapted and viable bacteria as claimed in Claim 24.

27. The additive food product as claimed in Claim 26, characterised in that it is a milk product and/or a beverage.

28. A manufacturing process for an additive food product as claimed in any one of Claims 26 or 27, characterised in that the liquid concentrate of adapted and viable bacteria is added to the food product at the end of the production line and preferably prior to packaging of the food product.